DNA polymerase eta is the sole contributor of A/T modifications during immunoglobulin gene hypermutation in the mouse.

Frédéric Delbos, Said Aoufouchi, Ahmad Faili, Jean-Claude Weill, Claude-Agnès Reynaud

DNA polymerase η is the sole contributor of A/T modifications during immunoglobulin gene hypermutation in the mouse

Frédéric Delbos,[1,2] Said Aoufouchi,[1,2] Ahmad Faili,[1,2] Jean-Claude Weill,[1,2] and Claude-Agnès Reynaud[1,2]

[1]Institut National de la Santé et de la Recherche Médicale U783 ("Développement du système immunitaire") and [2]Université Paris René Descartes, Faculté de Médecine René Descartes, Site Necker–Enfants Malades, 75730 Paris Cedex 15, France

Mutations at A/T bases within immunoglobulin genes have been shown to be generated by a repair pathway involving the DNA-binding moiety of the mismatch repair complex constituted by the MSH2–MSH6 proteins, together with DNA polymerase η (pol η). However, residual A/T mutagenesis is still observed upon inactivation in the mouse of each of these factors, suggesting that the panel of activities involved might be more complex. We reported previously (Delbos, F., A. De Smet, A. Faili, S. Aoufouchi, J.-C. Weill, and C.-A. Reynaud. 2005. *J. Exp. Med.* 201:1191–1196) that residual A/T mutagenesis in pol η–deficient mice was likely contributed by another enzyme not normally involved in hypermutation, DNA polymerase κ, which is mobilized in the absence of the normal polymerase partner. We report the complete absence of A/T mutations in MSH2–pol η double-deficient mice, thus indicating that the residual A/T mutagenesis in MSH2-deficient mice is contributed by pol η, now recruited by uracil *N*-glycosylase, the second DNA repair pathway involved in hypermutation. We propose that this particular recruitment of pol η corresponds to a profound modification of the function of uracil glycosylase in the absence of the mismatch repair complex, suggesting that MSH2–MSH6 actively prevent uracil glycosylase from error-free repair during hypermutation. pol η thus appears to be the sole contributor of A/T mutations in the normal physiological context.

CORRESPONDENCE
C.-A. Reynaud:
reynaud@necker.fr
OR
J.-C. Weill:
weill@necker.fr

Somatic hypermutation is a specific mutagenic process that can diversify the Ig genes at several stages of the B cell differentiation pathway: either outside an immune response, leading to repertoire diversification, or during antigen driven responses, leading to affinity maturation through the selection of B cells displaying better antigen-binding capacities (1).

Hypermutation is initiated by activation-induced cytidine deaminase (AID) through an enzymatic process that deaminates cytidines into uracils and is essentially, but not exclusively, targeted at the heavy and light chain V genes of the Ig locus (reference 2; for review see references 3, 4). However, mutations at the Ig locus are equally distributed at G/C and A/T bases, which requires that specific error-prone repair pathways process this C-focused lesion to broaden its mutation spectrum (5). In spite of the large number of repair factors able to handle uracils within the eukaryotic cell, only two of them have been shown to contribute to hypermutation: uracil *N*-glycosylase (UNG) and the DNA-binding moiety of the mismatch repair complex (MSH2–MSH6) (6–14). When these two factors are missing, the mutation pattern becomes the imprint of the sole AID action, the replication of uracils generated on both DNA strands resulting in C to T and G to A transitions at the sites of AID deamination (14).

Error-prone DNA polymerases, in particular those involved in the replicative bypass of DNA lesions, have been considered as prime candidates to generate mutations at A/T base pairs from the initial deamination event. The Ig gene mutation pattern of patients affected with the variant form of the xeroderma pigmentosum syndrome, a genetic disease corresponding to inactivation of DNA polymerase η (pol η) (15), was shown to be markedly, albeit

The online version of this article contains supplemental material.

variably, reduced in A/T mutagenesis (16–18). Inactivation of pol η in the mouse confirmed this phenotype and resulted in a more homogeneous reduction in A/T mutations (19–21). Similarly, inactivation of the MSH2 protein (or of its MSH6 and exonuclease-1 partners) in the mouse has been shown to result in a decreased mutation load at A/T bases (7–13). These observations have led to a model of hypermutation in which UNG-mediated repair would be responsible for most mutations at G/C bases, whereas the MSH2 pathway would generate the major part of A/T mutations (14).

But how is the residual A/T mutagenesis observed in both pol η or MSH2-deficient animals generated? We previously reported that, in *Polh*$^{-/-}$ mice, the A/T mutation pattern bears the signature of a different enzyme (19), most probably pol κ, which has an unusual bias toward transversions (22, 23). The absence of effect of pol κ deficiency on the Ig mutation pattern (24–26) strongly suggested that this enzyme is probably not a normal actor in hypermutation but would rather be recruited only in the absence of the regular polymerase partner. We show in this report that the residual A/T mutagenesis observed in MSH2-deficient mice is contributed by pol η, now driven by the UNG pathway, thus establishing pol η as the sole polymerase generating A/T mutations during the physiological mutation process. The recruitment of pol η by UNG is discussed in the context of the striking modification of the mutation pattern observed in MSH2-deficient animals, which leads us to propose a competitive function of mismatch repair that prevents uracil glycosylase from normal, error-free repair.

RESULTS AND DISCUSSION

The residual A/T mutagenesis observed in MSH2-deficient animals harbors the same overall pattern as the one observed in Ig genes of normal mice, which raises the possibility that it could be generated by pol η, recruited outside the mismatch repair pathway.

We therefore generated *Polh*$^{-/-}$*Msh2*$^{-/-}$ animals and collected a large mutation database from four to seven mice of the single- or double-deficient genotypes. We observed rather large interindividual variations in the mutation frequency, notably between two groups of wild-type mice originating from two separate rooms of the same animal facility (Table I and Fig. 1 A). Taking into account the mutation frequency of the pool of mice constituted by the wild-type littermates of the *Polh* heterozygous breeding, the mutation frequency was reduced at least two times in either MSH2- or pol η–MSH2–deficient mice. This reduction is mainly caused by the absence of highly mutated sequences (Fig. 1 C).

Previous studies have concluded, based on [^{3}H]thymidine incorporation after different mitogenic stimulations, that MSH2 deficiency did not result in a significant in vitro proliferative defect of splenic B cells, although isotype switching was reduced by two thirds (7, 27–30). Using carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling during in vitro stimulation of splenic B cells with either LPS or LPS plus IL-4, we observed no difference in proliferation of pol η–deficient B cells compared with controls, and only a marginal effect of MSH2 deficiency, which was, however, not manifest in the double *Pol η–Msh2*$^{-/-}$ context (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20062131/DC1).

pol η–MSH2–deficient mice show the same altered distribution of mutation along the Ig sequence as *Msh2*$^{-/-}$ mice do, with a striking clustering at a few G/C hotspot positions: seven hotspots concentrate more than half of the mutations in a sequence of 490 bp (Fig. 2). Most of them (except one, TGTT, at position 38 in the J_H4 intronic sequence, whose targeting is, however, less pronounced in the double-deficient background) correspond to a WGCW sequence, as described previously (W = A or T) (12), with either or both internal G and C bases targeted. One major hotspot present in wild-type mice (AGTT, at position 46) disappears in the absence of MSH2. Similar again to the MSH2-deficient genotype, a strong increase in transitions within G/C mutations is observed in the double-deficient context (Table II).

The main difference between MSH2 and pol η–MSH2–deficient animals resides in A/T mutations: whereas they represent 10% of mutations in *Msh2*$^{-/-}$ mice, they are totally absent in the double knockout mice, with the 4 A/T changes collected in a sample of 310 mutations corresponding to the background of the enzyme used for amplification (Fig. 1 B). It should be noted that the low mutation frequency linked with the MSH2 genetic defect (in the 2×10^{-3} range)

Table I. Somatic mutations in J_H4 intronic sequences (490 bp) from normal and mutant mice

	Controls		*pol η*$^{-/-}$	*Msh2*$^{-/-}$	*pol η*$^{-/-}$ × *Msh2*$^{-/-}$
	1 (4 mice)	2 (4 mice)	(7 mice)	(7 mice)	(4 mice)
Number of sequences	112	89	193	208	331
Total length sequenced (bp)	54,880	43,610	94,570	101,920	162,190
Unmutated sequences (percentage)	25	48	31	50	60
Total number of mutations	624	251	508	242	310
Number of deletions and insertions	8	3	6	4	5
Mutation frequency per total sequences (per 100 bp)	1.15	0.58	0.54	0.24	0.19
Mutation frequency per mutated sequences (per 100 bp)	1.54	1.13	0.78	0.48	0.48

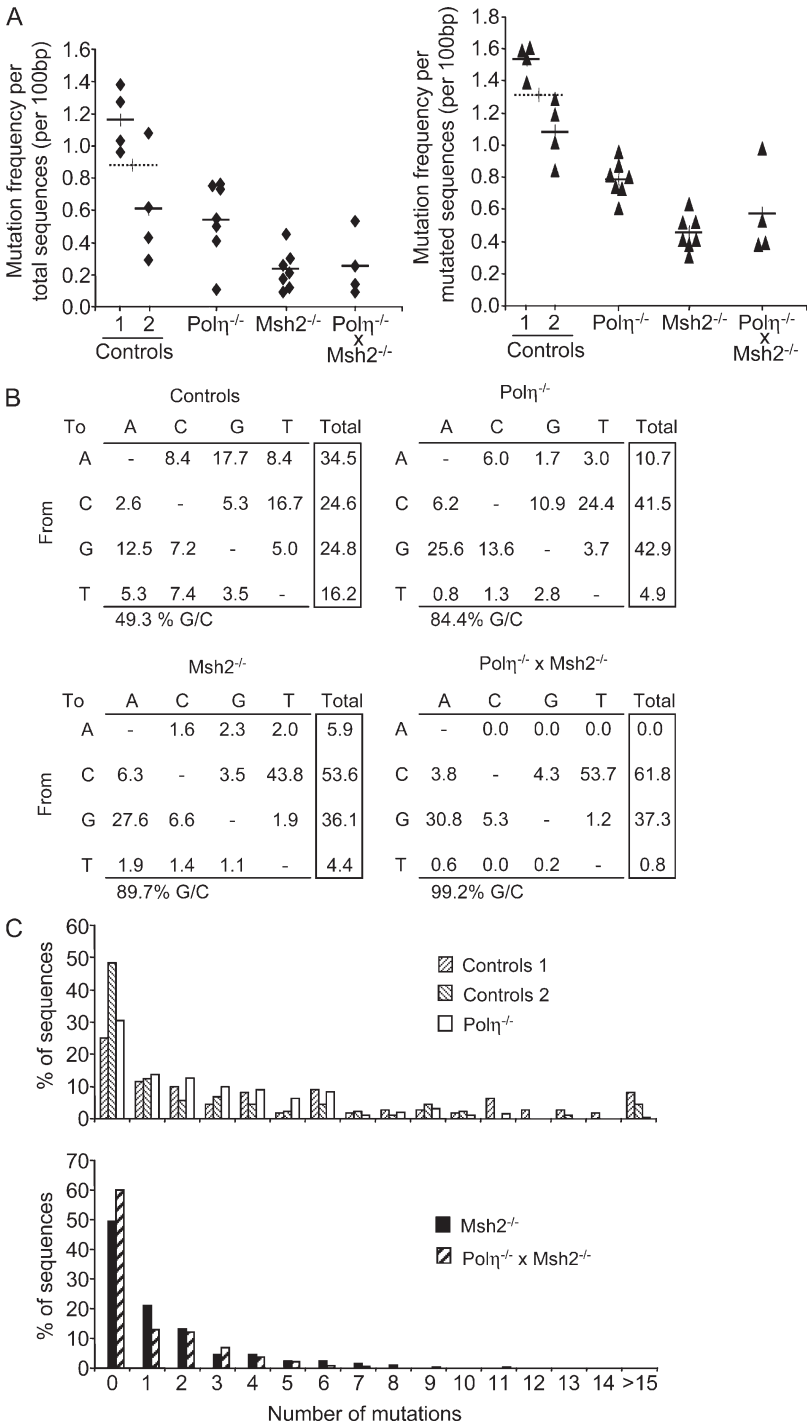

Figure 1. Analysis of mutations in rearranged J_H4 intronic sequences isolated from Peyer's patches of controls and pol η–, MSH2- and pol η–MSH2–deficient mice. (A) Average mutation frequency per individual mouse, expressed relative to total sequences (left) or to mutated sequences (right). The mean values are represented by a horizontal bar. Controls (2) represent wild-type littermates of the *Polh* heterozygous breedings, whereas controls (1) come from a different module from the same animal facility (the dotted bar is the mean between the two sets of controls). These mean values differ slightly from the ones listed in Table I, which represent the average mutation frequency of pooled sequences. (B) Pattern of nucleotide substitution in the four different genotypes of mice. Values are expressed as the percentage of total mutations after correction for base composition. (C) Accumulation of mutations in individual J_H4 intronic sequences. The number of sequences harboring a defined number of mutations relative to the total number of sequences is represented. MSH2-proficient (top) and MSH2-deficient (bottom) backgrounds are shown. All mutations are listed along the J_H4 intronic sequence in Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20062131/DC1.

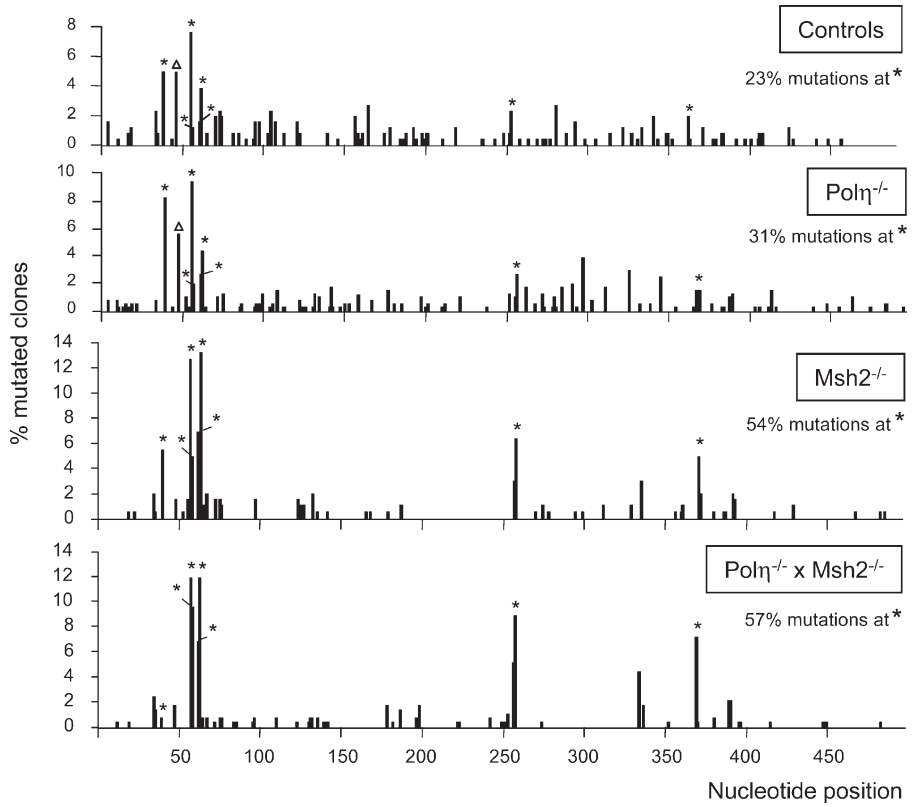

Figure 2. Hotspot clustering of mutations in MSH2-deficient backgrounds. The distribution of mutations at G/C bases along the J_H4 intronic sequence is represented for the four different genetic backgrounds analyzed. The percentage of total mutations represented by the seven major hotspots observed in the *Msh2*$^{-/-}$ background (defined arbitrarily as a position mutated in 5% or more of sequences) is calculated for each genotype (marked by asterisks). These seven mutation hotspots occur in the following sequence context (described in their 5′ to 3′ order along the J_H4 sequence, with the mutated base underlined and the position of the first base of the motif numbered from the J_H4 intronic border): TGTT (position 38), AGCA (position 55), TGCA (position 60), TGCT (position 251), and AGCA (position 362). One hotspot marked with an open triangle (AGTT, at position 46) is restricted to the MSH2-proficient background.

obviously requires high fidelity enzymes to collect mutation samples in which the contribution of the amplification step does not exceed a few percent (2–3% in this experiment, with ∽60% of them being As or Ts) (31).

It thus appears that pol η is responsible for the residual A/T mutagenesis observed in MSH2-deficient animals by being involved in a mutagenic DNA synthesis that now appears to be driven by the UNG pathway. Taking into account our previous data suggesting that, in pol η–deficient mice, the A/T mutations observed are likely contributed by pol κ recruited in a back-up function, we therefore conclude that, in the normal physiological situation, pol η is the sole polymerase required for A/T mutagenesis at the Ig locus. pol η probably generates some G/C mutations as well, as its mutation spectrum in vitro affects G/C bases in a proportion of ∽20%, with two thirds of them being transitions and one third being transversions (32). These numbers fit remarkably well with the 3% of transversion mutations at G/C positions observed in UNG-deficient mice (6); indeed, this suggests that 10% of G/C mutations could be generated by pol η via the MSH2 pathway, with one third of them being discernable as transversions in the UNG-deficient context.

Is the small contribution of pol η via the UNG pathway observed in MSH2-deficient animals a physiological process? Although this question might not be easily answered, we would like to argue that the overall mutation pattern of MSH2-deficient animals corresponds to a major alteration of the repair pathways involved, a situation for which, surprisingly, no comprehensive explanation has been brought so far. The seminal work of Rada et al. (14) has shown that the mutation pattern of UNG–MSH2–deficient animals reflects the simple footprint of AID deamination and that it is close, in terms of targeting, to the G/C mutation pattern of wild-type animals. It is also quantitatively similar, at least at the V locus, and this striking observation that suggests that both UNG and MSH2 pathways are processing uracils mainly in an error-prone mode. It thus follows that the large decrease in mutation frequency, as well as the increased targeting of specific hotspots observed in the MSH2-deficient background, must be caused by an increased error-free repair of deaminated cytosines by the UNG pathway rather than by

Table II. Pattern of nucleotide changes in J_H4 intronic sequences of normal and mutant mice

			Within G/C			Within A/T		
			Trans.	Transv.		Trans.	Transv.	
	GC:AT	Transitions: transversions	G/A C/T	G/T C/A	G/C C/G	A/G T/C	A/T T/A	A/C T/G
Controls	49.3:50.7	54.3:45.7	59.1	15.6	25.3	49.5	27.0	23.5
pol $\eta^{-/-}$	84.4:15.6	53.0:47.0	59.3	11.7	29.0	18.8	24.7	56.5
Msh2$^{-/-}$	89.7:10.3	75.1:24.9	79.6	9.2	11.2	35.6	37.8	26.6
pol $\eta^{-/-} \times$ *Msh2*$^{-/-}$	99.2:0.8	84.6:15.4	85.3	5.0	9.7	0	75.0	25.0

modulation of AID targeting by the MSH2–MSH6 complex, as proposed (12, 33). The mutation hotspots that emerge could correspond to deamination sites at which the residence time of AID would be increased (possibly through their symmetrical WGCW structure that would result in a stronger binding and/or an inefficient displacement by the sole UNG enzyme), thus preventing repair at those sites. The AGTT hotspot whose occurrence is restricted to the MSH2-proficient background would not provide such strong binding and would be more easily repaired. Error-free repair or ignorance would then be the two major outcomes of AID-induced cytidine deamination in the MSH2-deficient background, resulting in the drop of mutation frequency and the increase in transitions at G/C bases. A small fraction of lesions handled by the UNG pathway would generate abasic sites, leading to transversions when replicated over, or would recruit pol η in an error-prone short patch repair (Fig. 3).

Collectively, these data emphasize the very dissymmetrical role that the UNG and MSH2 pathways play in the hypermutation process. UNG deficiency affects mainly the nature of G/C mutations, which are biased almost exclusively toward transitions, while having only a moderate impact on the A/T mutagenesis driven by MSH2 (6, 34) and none on the mutation frequency. In contrast, the absence of mismatch repair leads to a stronger modification of the Ig gene mutation pattern, affecting not only mutations at A/T bases, as would be expected from a function symmetrical to the one of UNG, but also the overall mutation frequency and, within G/C mutations, the proportion of transitions as well as their distribution along the Ig sequence. Overall, this suggests that, although the function of MSH2–MSH6 is relatively independent from UNG, the mismatch repair complex strongly impinges on the role of UNG during hypermutation by preventing it to perform its natural error-free repair function.

The behavior of UNG in the mismatch repair–deficient context strikingly mirrors the effect of the deliberate overexpression of another uracil glycosylase, SMUG1, in a UNG–MSH2–deficient background, which results in a major error-free repair accompanied by a smaller error-prone activity generating A/T mutations and G/C transversions (35).

In conclusion, the exclusive formation of A/T mutations by pol η allows the formulation of a simplified scheme for the role of translesional polymerases in hypermutation (Fig. 3). pol η would be recruited by the MSH2–MSH6 complex outside of the S phase to permit U/G mismatch recognition, and would mostly generate A/T but also a small amount of

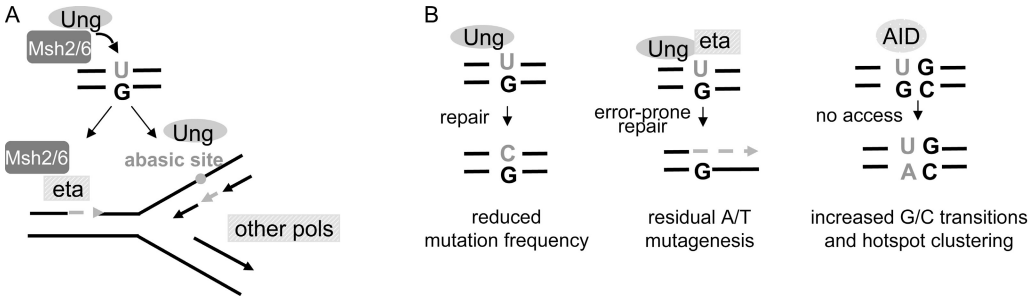

Figure 3. Impact of the MSH2–MSH6 complex on UNG activity during Ig gene hypermutation. (A) A simplified scheme of hypermutation. UNG would be prevented from performing error-free repair in the presence of MSH2–MSH6 and would generate mainly abasic sites upon uracil recognition. These DNA lesions would be copied by a set of translesional DNA polymerases (among which are Rev1, Rev3, and possibly pol θ [references 36–40], albeit the contribution of this latter enzyme was recently shown to be less likely [reference 41]), acting in S phase in their function of lesion bypass. MSH2–MSH6 would recruit pol η in an error-prone short patch synthesis of the uracil-containing strand, most likely in G1 (reference 42). (B) Outline of a possible altered behavior of UNG in the absence of MSH2 and of its consequences on hypermutation in *Msh2*$^{-/-}$ mice. (left) Increase of error-free repair (resulting in a reduced mutation frequency). (middle) Recruitment of pol η for an error-prone short patch repair (residual A/T mutagenesis). (right) Inefficient displacement of AID (increase in transitions at G/C), in particular at WGCW sites (increased focusing of mutations at specific hotspot positions).

G/C mutations in an error-prone short patch repair process that appears thus far restricted to B cells. In contrast, most G/C mutations would be generated by several translesion DNA polymerases (33–38), whose exact number remains to be established, acting during replication in their function of lesion bypass by copying abasic sites generated by UNG, which is diverted by MSH2 from its error-free function. In such a model of hypermutation, and in contrast to their physiological repair function, UNG would trigger a translesional process at the replication fork, whereas MSH2–MSH6 would induce a base excision repair outside replication, most probably in the G1 phase of the cell cycle. The involvement of pol η in an error-prone process, whether driven by MSH2 during physiological hypermutation or by uracil glycosylases in specific experimental settings, thus appears to be a B cell–specific event whose biochemical basis remains to be established.

MATERIALS AND METHODS

Generation of gene-targeted mice. *Polh-Msh2* double knockout mice were obtained by breeding the previously described *Polh*$^{-/-}$ mouse strain (19) with *Msh2*$^{-/-}$ animals provided by Hein te Riele (The Netherlands Cancer Institute, Amsterdam, Netherlands). Both genes are located distantly enough on chromosome 17 to allow the efficient recovery of their combined inactivation by mouse breeding. All MSH2-deficient animals selected for analysis were devoid of overt tumors. Generation of gene-targeted mice and breeding was performed by the Service d'Expérimentation Animale et de Transgénèse. Experiments were performed according to the Institut national de la santé et de la recherche médicale guidelines for laboratory animals and were approved by the Scientific Committee of the Necker Animal Facility.

B cell proliferation assays. Splenic B cells were isolated from individual 2–3-mo-old mice by negative selection using the Mouse B Cell Isolation Kit (Miltenyi Biotec). Purified B cells were labeled with 5 μM CFSE, according to the manufacturer's instructions (Vybrant CFDA SE Cell Tracer Kit; Invitrogen), before stimulation with either 20 μg/ml *Escherichia coli* LPS (serotype 055:B5; Sigma-Aldrich) or 20 μg LPS plus 10 ng/ml IL-4 (Preprotech). CFSE-labeled cells were analyzed after 3 d of stimulation, with forward scatter gating on live cells.

Sequence analysis. B220^{+}PNA^{high} B cells were isolated from Peyer's patches of 4–6-mo-old animals as previously described (8). The J_H4 intron flanking rearranged V_H sequences was amplified using a mixture of five V_H primers designed to amplify most of the mouse V_H families and a downstream primer allowing the determination of 490 bp of noncoding sequences, as reported previously (19).

Online supplemental material. Fig. S1 shows in vitro proliferation of splenic B cells from wild-type, *Polh*$^{-/-}$, *Msh2*$^{-/-}$, and *Msh2*$^{-/-}$*Polh*$^{-/-}$ mice. Fig. S2 depicts the distribution of mutations along the J_H4 intronic sequence in Peyer's patch PNA^{high} B cells from wild-type, *Polh*$^{-/-}$, *Msh2*$^{-/-}$, and *Msh2*$^{-/-}$*Polh*$^{-/-}$ mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20062131/DC1.

We thank Jérome Mégret for performing cell sorting and the Service d'Expérimentation Animale et de Transgénèse for the generation and handling of gene-targeted animals. We thank Barbara Bertocci for advice concerning cell proliferation assays.

This work was supported by the Ligue Nationale Contre le Cancer (Equipe labelisée) and the Fondation Princesse Grace de Monaco.

The authors have no conflicting financial interests.

Submitted: 4 October 2006
Accepted: 4 December 2006

REFERENCES

1. Longo, N.S., and P.E. Lipsky. 2006. Why do B cells mutate their immunoglobulin receptors? *Trends Immunol.* 27:374–380.
2. Muramatsu, M., K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Honjo. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell.* 102:553–563.
3. Longerich, S., U. Basu, F.W. Alt, and U. Storb. 2006. AID in somatic hypermutation and class switch recombination. *Curr. Opin. Immunol.* 18:164–174.
4. Odegard, V.H., and D.G. Schatz. 2006. Targeting of somatic hypermutation. *Nat. Rev. Immunol.* 6:573–583.
5. Reynaud, C.-A., S. Aoufouchi, A. Faili, and J.-C. Weill. 2003. What role for AID: mutator, or assembler of the immunoglobulin mutasome? *Nat. Immunol.* 4:631–638.
6. Rada, C., G.T. Williams, H. Nilsen, D.E. Barnes, T. Lindahl, and M.S. Neuberger. 2002. Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. *Curr. Biol.* 12:1748–1755.
7. Rada, C., M.R. Ehrenstein, M.S. Neuberger, and C. Milstein. 1998. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity.* 9:135–141.
8. Frey, S., B. Bertocci, F. Delbos, L. Quint, J.-C. Weill, and C.-A. Reynaud. 1998. Mismatch repair deficiency interferes with the accumulation of mutations in chronically stimulated B cells and not with the hypermutation process. *Immunity.* 9:127–134.
9. Phung, Q.H., D.B. Winter, A. Cranston, R.E. Tarone, V.A. Bohr, R. Fishel, and P.J. Gearhart. 1998. Increased hypermutation at G and C nucleotides in immunoglobulin variable genes from mice deficient in the MSH2 mismatch repair protein. *J. Exp. Med.* 187:1745–1751.
10. Wiesendanger, M., B. Kneitz, W. Edelmann, and M.D. Scharff. 2000. Somatic hypermutation in MutS homologue (MSH)3-, MSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2-MSH6 heterodimer in modulating the base substitution pattern. *J. Exp. Med.* 191:579–584.
11. Bardwell, P.D., C.J. Woo, K. Wei, Z. Li, A. Martin, S.Z. Sack, T. Parris, W. Edelman, and M.D. Scharff. 2004. Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat. Immunol.* 5:224–229.
12. Martomo, S.A., W.W. Yang, and P.J. Gearhart. 2004. A role for Msh6 but not Msh3 in somatic hypermutation and class switch recombination. *J. Exp. Med.* 200:61–68.
13. Li, Z., S.J. Scherer, D. Ronai, M.D. Iglesias-Ussel, J.U. Peled, P.D. Bardwell, M. Zhuang, K. Lee, A. Martin, W. Edelmann, and M.D. Scharff. 2004. Examination of Msh6- and Msh3-deficient mice in class switching reveals overlapping and distinct roles of MutS homologues in antibody diversification. *J. Exp. Med.* 200:47–59.
14. Rada, C., J.M. Di Noia, and M.S. Neuberger. 2004. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T focused phase of somatic mutation. *Mol. Cell.* 16:163–171.
15. Masutani, C., R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio, and F. Hanaoka. 1999. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature.* 399:700–704.
16. Zeng, X., D.B. Winter, C. Kasmer, K.H. Kraemer, A.R. Lehmann, and P.J. Gearhart. 2001. DNA polymerase eta is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. *Nat. Immunol.* 2:537–541.
17. Faili, A., S. Aoufouchi, S. Weller, F. Vuillier, A. Stary, A. Sarasin, C.-A. Reynaud, and J.-C. Weill. 2004. DNA polymerase η is involved in hypermutation occurring during immunoglobulin class switch recombination. *J. Exp. Med.* 199:265–270.
18. Zeng, X., G.A. Negrete, C. Kasmer, W.W. Yang, and P.J. Gearhart. 2004. Absence of DNA polymerase η reveals targeting of C mutations on the nontranscribed strand in immunoglobulin switch regions. *J. Exp. Med.* 199:917–924.
19. Delbos, F., A. De Smet, A. Faili, S. Aoufouchi, J.-C. Weill, and C.-A. Reynaud. 2005. Contribution of DNA polymerase η to

immunoglobulin gene hypermutation in the mouse. *J. Exp. Med.* 201:1191–1196.
20. Martomo, S.A., W.W. Yang, R.P. Wersto, T. Ohkumo, Y. Kondo, M. Yokoi, C. Masutani, F. Hanaoka, and P.J. Gearhart. 2005. Different mutation signatures in DNA polymerase eta- and MSH6-deficient mice suggest separate roles in antibody diversification. *Proc. Natl. Acad. Sci. USA.* 102:8656–8661.
21. Martomo, S.A., W.W. Yang, A. Vaisman, A. Maas, M. Yokoi, J.H. Hoeijmakers, F. Hanaoka, R. Woodgate, and P.J. Gearhart. 2006. Normal hypermutation in antibody genes from congenic mice defective for DNA polymerase iota. *DNA Repair (Amst.).* 5:392–398.
22. Ohashi, E., K. Bebenek, T. Matsuda, W.J. Feaver, V.L. Gerlach, E.C. Friedberg, H. Ohmori, and T.A. Kunkel. 2000. Fidelity and processivity of DNA synthesis by DNA polymerase kappa, the product of the human DINB1 gene. *J. Biol. Chem.* 275:39678–39684.
23. Zhang, Y., F. Yuan, H. Xin, X. Wu, D.K. Rajpal, D. Yang, and Z. Wang. 2000. Human DNA polymerase kappa synthesizes DNA with extraordinarily low fidelity. *Nucleic Acids Res.* 28:4147–4156.
24. Schenten, D., V.L. Gerlach, C. Guo, S. Velasco-Miguel, C.L. Hladik, C.L. White, E.C. Friedberg, K. Rajewsky, and G. Esposito. 2002. DNA polymerase kappa deficiency does not affect somatic hypermutation in mice. *Eur. J. Immunol.* 32:3152–3160.
25. Shimizu, T., Y. Shinkai, T. Ogi, H. Ohmori, and T. Azuma. 2003. The absence of DNA polymerase kappa does not affect somatic hypermutation of the mouse immunoglobulin heavy chain gene. *Immunol. Lett.* 86:265–270.
26. Shimizu, T., T. Azuma, M. Ishiguro, N. Kanjo, S. Yamada, and H. Ohmori. 2005. Normal immunoglobulin gene somatic hypermutation in Pol kappa-Pol iota double-deficient mice. *Immunol. Lett.* 98:259–264.
27. Schrader, C.E., W. Edelmann, R. Kucherlapati, and J. Stavnezer. 1999. Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes. *J. Exp. Med.* 190:323–330.
28. Schrader, C.E., J. Vardo, and J. Stavnezer. 2003. Mlh1 can function in antibody class switch recombination independently of Msh2. *J. Exp. Med.* 197:1377–1383.
29. Martin, A., Z. Li, D.P. Lin, P.D. Bardwell, M.D. Iglesias-Ussel, W. Edelmann, and M.D. Scharff. 2003. Msh2 ATPase activity is essential for somatic hypermutation at A-T base pairs and for efficient class switch recombination. *J. Exp. Med.* 198:1171–1178.
30. Vora, K.A., K.M. Tumas-Brundage, V.M. Lentz, A. Cranston, R. Fishel, and T. Manser. 1999. Severe attenuation of the B cell immune response in Msh2-deficient mice. *J. Exp. Med.* 189:471–482.
31. Biles, B.D., and B.A. Connolly. 2004. Low-fidelity *Pyrococcus furiosus* DNA polymerase mutants useful in error-prone PCR. *Nucleic Acids Res.* 32:e176.
32. Matsuda, T., K. Bebenek, C. Masutani, I.B. Rogozin, F. Hanaoka, and T.A. Kunkel. 2001. Error rate and specificity of human and murine DNA polymerase eta. *J. Mol. Biol.* 312:335–346.
33. Li, Z., C. Zhao, M.D. Iglesias-Ussel, Z. Polonskaya, M. Zhuang, G. Yang, Z. Luo, W. Edelmann, and M.D. Scharff. 2006. The mismatch repair protein Msh6 influences the in vivo AID targeting to the Ig locus. *Immunity.* 24:393–403.
34. Longerich, S., A. Tanaka, G. Bozek, D. Nicolae, and U. Storb. 2005. The very 5′ end and the constant region of Ig genes are spared from somatic mutation because AID does not access these regions. *J. Exp. Med.* 202:1443–1454.
35. Di Noia, J.M., C. Rada, and M.S. Neuberger. 2006. SMUG1 is able to excise uracil from immunoglobulin genes: insight into lutation versus repair. *EMBO J.* 25:585–595.
36. Diaz, M., L.K. Verkoczy, M.F. Flajnik, and N.R. Klinman. 2001. Decreased frequency of somatic hypermutation and impaired affinity maturation but intact germinal center formation in mice expressing antisense RNA to DNA polymerase zeta. *J. Immunol.* 167:327–335.
37. Zan, H., A. Komori, Z. Li, A. Cerutti, A. Schaffer, M.F. Flajnik, M. Diaz, and P. Casali. 2001. The translesion DNA polymerase zeta plays a major role in Ig and bcl-6 somatic hypermutation. *Immunity.* 14:643–653.
38. Jansen, J.G., P. Langerak, A. Tsaalbi-Shtylik, P. van den Berk, H. Jacobs, and N. de Wind. 2006. Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. *J. Exp. Med.* 203:319–323.
39. Zan, H., N. Shima, Z. Xu, A. Al-Qahtani, I.A.J. Evinger III, Y. Zhong, J.C. Schimenti, and P. Casali. 2005. The translesion DNA polymerase theta plays a dominant role in immunoglobulin gene somatic hypermutation. *EMBO J.* 24:3757–3769.
40. Masuda, K., R. Ouchida, A. Takeuchi, T. Saito, H. Koseki, K. Kawamura, M. Tagawa, T. Tokuhisa, T. Azuma, and J. O-Wang. 2005. DNA polymerase theta contributes to the generation of C/G mutations during somatic hypermutation of Ig genes. *Proc. Natl. Acad. Sci. USA.* 102:13986–13991.
41. Masuda, K., R. Ouchida, M. Hikida, M. Nakayama, O. Ohara, T. Kurosaki, and J. O-Wang. 2006. Absence of DNA polymerase theta results in decreased somatic hypermutation frequency and altered mutation patterns in Ig genes. *DNA Repair (Amst.).* 5:1384–1391.
42. Wilson, T.M., A. Vaisman, S.A. Martomo, P. Sullivan, L. Lan, F. Hanaoka, A. Yasui, R. Woodgate, and P.J. Gearhart. 2005. MSH2–MSH6 stimulates DNA polymerase η, suggesting a role for A:T mutations in antibody genes. *J. Exp. Med.* 201:637–645.